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Research Note

Histopathology of *Oligacanthorhynchus tortuosa* (Oligacanthorhynchidae) Infection in the Virginia Opossum (*Didelphis virginiana*)

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ABSTRACT: *Oligacanthorhynchus tortuosa*, a common acanthocephalan of the Virginia opossum (*Didelphis virginiana*) in North America, has been reported to be associated with large, nodule-like lesions at points of attachment of the proboscides. Three lesions resulting from the attachment of individuals of *O. tortuosa*, 1 each from 3 infected opossums, were prepared for histological examination to further characterize histopathologic changes elicited by this parasite. Histologically, lesions involved the mucosa, submucosa, and muscularis. The proboscides were contained within abscesses characterized by necrotic debris interspersed with many pycnotic nuclei. The abscesses were approximately 1.4 mm in diameter and were surrounded by regions of dense connective tissue (collagen), approximately 142 μ m wide. The bands of dense connective tissue were surrounded by regions of active fibroblast and fibrocyte proliferation, approximately 169 μ m wide, in which evidence of collagen synthesis was observed. Both longitudinal and smooth muscle layers of the muscularis had been completely destroyed in the area of the lesion. Absence of polymorphonuclear leukocytes were indicative of chronic lesions. Histopathologic changes elicited by *O. tortuosa* include chronic inflammatory response to mechanical trauma resulting from injury caused by the proboscis with subsequent fibrosis and nodule formation.

KEY WORDS: histopathology, *Oligacanthorhynchus tortuosa*, *Didelphis virginiana*, opossum, Acanthocephala.

Oligacanthorhynchus tortuosa, a common acanthocephalan of the Virginia opossum (*Didelphis virginiana*) in North America, has been reported to be associated with large, nodule-like lesions at points of attachment of proboscides. *Oligacanthorhynchus tortuosa* is represented by large worms with females achieving lengths of up to 350 mm (Richardson, unpubl. data). The

globular proboscis bears 6 spiral rows of 6 hooks each and has a length of 0.22–0.23 mm and width of 0.23–0.29 mm (Van Cleave, 1953). Leidy (1850) reported a specimen of *O. tortuosa* as having the anterior 3 lines of its length buried in an oval tumor, 4 lines in diameter, in the mesentery of an opossum. Based on this statement, Van Cleave (1924) concluded that the worm had penetrated the intestinal wall, entered the body cavity, and attached to the mesentery. Feldman et al. (1972) reported severe ulcerative lesions evoked at points of attachment of unidentified acanthocephalans from opossums. Brief description and a photomicrograph (Feldman et al., 1972) suggest that these specimens were *O. tortuosa*. Richardson et al. (1992) reported 2 poorly developed individuals of *O. tortuosa* from the small intestine of a raccoon (*Procyon lotor*) that caused "severe lesions" at points of attachment; however, histological examination was not conducted. The only histological examination of lesions caused by *O. tortuosa* was conducted by Babero (1957), who reported elevated nodules over the serosal surface of the small intestines of 2 Illinois opossums having a base diameter of 2–7 mm. He reported the nodules to have a bright red appearance due to congestion of intestinal blood vessels. Histologically, lesions reported by Babero (1957) resulted in complete mechanical destruction of the mucosal and submucosal layers with some focal atrophy and necrosis of the muscularis. He further noted limited leukocytic infiltration and some pigment deposition. Babero (1960) examined opossums from Georgia

infected with *O. tortuosa* in which no such hemorrhagic lesions were observed.

The purpose of this investigation was to further characterize lesions resulting from *O. tortuosa* infections in the Virginia opossum.

Three lesions resulting from the attachment of individuals of *O. tortuosa*, 1 each from 3 opossums, were prepared for histological examination. Material was obtained from opossums collected in the course of a survey of Acanthocephala of opossums from Arkansas, results of which were reported by Richardson (1993). All lesions examined were caused by mature worms. Immediately after sacrificing the opossum, the small intestine was examined for nodules on the serosal surface, then removed and longitudinally dissected. Mature worms were severed so as to leave the proboscis intact and undisturbed in the lesion. The lesion along with normal tissue immediately surrounding the lesion was excised and placed directly into Bouin's fixative.

After fixing in Bouin's solution for 24 hr, tissues were stored in 70% ethanol. Tissues were dehydrated by treating in a graded series of ethanol. Tissues were cleared in toluene, infiltrated and embedded in paraffin blocks, and sectioned at a thickness of 5–7 μm using a rotary microtome. Ribbons were attached to slides with albumin and stained following Masson's trichrome technique as described by Luna (1968). Two percent light green was substituted in place of aniline blue to enhance staining of connective tissue. Stained sections were mounted in Canada balsam and examined using light microscopy.

Worms were restricted to the anterior $\frac{1}{2}$ of the small intestine, with most occurring in the anterior $\frac{1}{3}$. Grossly, hard, white nodules were apparent on the serosal surface of the intestine corresponding to points of worm attachment. Proboscides were firmly embedded in the intestinal wall resulting in nodule formation; however, there was no apparent evidence of hemorrhage as observed grossly by Babero (1957). At the base, nodules ranged from approximately 1 to 5 mm in diameter.

Lesions involved the mucosa, submucosa, and muscularis; however, the serosa was intact and apparently unaffected (Fig. 1). Proboscides were contained within abscesses characterized by necrotic debris interspersed with many pycnotic nuclei, particularly abundant around the periphery of abscesses. Small aggregations of collagen were present. No evidence of recent hemorrhage was observed. Abscesses, which were approxi-



Figure 1. Photomicrograph of a cross-section of a lesion elicited by *Oligacanthorhynchus tortuosa* in the intestine of a Virginia opossum (*Didelphis virginiana*) showing proboscis (arrow), necrotic abscess (asterisk), ring of collagen (C), region of active fibrocyte proliferation (arrowhead), muscularis (M), normal submucosa (Sm), serosal side (S), and luminal side (L). Scale bar = 200 μm . Figure 1 appeared in *Foundations of Parasitology*, 5th edition, Wm. C. Brown, Publishers, and is used here with permission of the company.

mately 1.14 mm in diameter, were surrounded by regions of dense connective tissue (collagen), approximately 142 μm wide. These regions of dense connective tissue, which appeared to have effectively contained the abscesses, were interspersed with small numbers of fibrocytes and fibroblasts. Spaces were noted between strands of collagen, many of which appeared to be lymphatics. Bands of dense connective tissue were surrounded by regions of active fibroblast and fibrocyte proliferation, approximately 169 μm wide, in which evidence of collagen synthesis could be observed. Within these regions, occasional plasma cells, lymphocytes, and mast cells were observed (approximately $1/0.133 \text{ mm}^2$ [$\times 40$ field]). These areas were infiltrated with many blood vessels and lymphatics. Lumina of arterioles were occluded by contraction of smooth

muscle in the arteriole wall. Abscesses along with bands of connective tissue resulted in drastic enlargement of the submucosa to over 7 times its normal width, resulting in formation of the grossly observable nodule. Width of the true submucosal region of unaffected tissue was approximately 240 μm , whereas that of affected regions was 1.87 mm. In the regions of the lesions, both longitudinal and smooth muscle layers of the muscularis had been completely eroded; however, the serosa remained intact and appeared to be unaffected. No evidence of hypertrophy of any of the muscular layers in the vicinity of the lesions was observed. The mucosa and muscularis mucosae appeared to be intact on luminal sides of the lesions except for entry points of worms, with thinning of the muscularis mucosae as an apparent result of stretching.

Histological series from which these data were obtained were deposited in the Harold W. Manter Laboratory, University of Nebraska, Lincoln, Nebraska, and given accession Nos. HWML 37832–37834.

Absence of polymorphonuclear leukocytes was indicative of chronic lesions. Observable pathology may be solely accounted for by mechanical damage resulting in subsequent fibrous nodule formation. These findings corroborate the synopsis given by Nicholas (1967), who summarized typical pathology of acanthocephalan infection as traumatic injury as a result of the proboscis penetrating deeply into the gut wall leading to an inflammatory response with cellular infiltration and the eventual formation of a dense fibrous nodule around the proboscis. Severe pathological manifestations associated with acanthocephalan infections often appear to be a result of peritonitis caused by perforation of the serosa by the proboscis (Stunkard, 1965; Schmidt, 1972). Apparently this phenomenon was observed by Leidy (1850) for *O. tortuosa*. This is likely considering the extent of mechanical damage found in the present study, including complete destruction of the muscularis.

Lesions examined in this study were similar to those described by Nelson and Nickol (1986) from domestic swine experimentally infected with *Macracanthorhynchus hirudinaceus*, which also had nodule formation as a result of an increase in size of the submucosa. Nelson and Nickol (1986) interpreted the preponderance of monocytes and lymphocytes, fibrosis, and neovascularization as evidence of a chronic lesion.

Extensive fibrosis suggests that frequent move-

ment of the worm does not occur after it has established and embedded its proboscis into the intestinal wall, unlike *Moniliformis moniliformis* in rats, which was found to attach superficially, penetrating only the mucosa and tunica propria, with no fibrosis (Taraschewski et al., 1989). Taraschewski et al. (1989) interpreted these lesions as evidence that worms frequently changed their sites of attachment.

Pathologic changes induced by *O. tortuosa* in the opossum were similar, with notable exceptions, to those elicited by *M. hirudinaceus* and *M. ingens*, which also belong to the family Oligacanthorhynchidae, in swine and raccoons, respectively (Nelson and Nickol, 1986). Nelson and Nickol (1986) reported extensive eosinophil proliferation and hypertrophy of the muscularis in raccoons infected with *M. ingens* and congregations of tissue macrophages, monocytes, and plasma cells in swine infected with *M. ingens*. Such a pronounced cellular response was not associated with *O. tortuosa* infections in the opossum. Additionally, hypertrophy of the muscularis was not observed. Instead, the muscularis was virtually completely destroyed and had been replaced by connective tissue. Both of these differences may possibly be accounted for by duration of infection. The observations of Nelson and Nickol (1986) were based on lesions from a raccoon infected with *M. ingens*, which was killed 63 days postinfection, and swine killed 3, 7, and 14 days after experimental infection with *M. ingens*. The duration of infection was not given for *M. hirudinaceus* in swine. It is possible that the reduced cellular response and pronounced erosion of the muscularis with subsequent fibrosis in the present study, which are characteristic of chronic inflammation (McCutcheon, 1948), was a result of increased duration of infection. The lack of hemorrhage, as described by Babero (1957), is further evidence of the chronicity of the lesions.

Pronounced absence of polymorphonuclear leukocytes suggests lack of a specific immune response to the presence of *O. tortuosa* with the resultant inflammatory response and subsequent fibrosis appearing to adequately contain the infection. Absence of polymorph proliferation suggests a low degree of pathogenicity elicited by this parasite. One opossum examined was infected with 99 *O. tortuosa*, mostly adults, and exhibited no overt signs of illness. Histopathologic changes elicited by *O. tortuosa* may be generalized as a chronic inflammatory response to

mechanical trauma resulting from injury caused by the proboscis, with subsequent fibrosis and nodule formation, not unlike the inflammatory response elicited by an inanimate irritating body as described by McCutcheon (1948).

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